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RESEARCH PAPER

Soluble organic nitrogen in forest soils of northeast China

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Abstract: Soluble organic nitrogen (SON) is recognized as a sensitive indicator of soil nitrogen status. The present work was conducted in the temperate forests of northeast China where soils are typically characterized by high organic matter and high organic nitrogen content, and soil sampling was made in early spring just after the freeze-thaw period. The water extracted SON pools in the organic layer of forest soils were measured within the range from 156.0 mg·kg^{-1} to 292.6 mg·kg^{-1}, a similar magnitude of salt solution extracted SON pools reported in literatures. However, the water soluble SON pools in 0–15 cm mineral soils in present study were much higher (3–10 times) than any other reports, ranging from 58.6 mg·kg^{-1} to 125.2 mg·kg^{-1}. Water soluble SON varied markedly among the soils under different forests and at different sites. The SON in water extracts were positively and significantly correlated to soil organic matter and total nitrogen contents, but negatively correlated to microbial biomass nitrogen (MBN). The reasons of the abnormally large SON pools and the negative correlations between SON and MBN in the 0–15cm mineral soils in this study were specially discussed.

Keywords: soluble organic nitrogen; microbial nitrogen; forest soil; temperate forest; Northeast China

Introduction

Forest soils are usually covered with litter layer of high organic matter content, which results in high concentrations of soluble organic N (SON) or dissolved organic N (DON) (Magill et al. 2000; Qualls et al. 2000; Zhong and Makeschin 2003). Many studies have demonstrated that DON can be a main pathway for N loss in forest soils (Andersson et al. 2000), and SON represents major inputs of N to surface water in forested watersheds and affects water quality (Currie et al. 1996; Hedin et al. 1995; Qualls et al. 2000; Yu et al. 2003). In the past few years, results from a number of studies have even confirmed that some plants (particularly those associated with mycorrhizae) are able to directly absorb certain fractions of SON (free amino acids) (Jones et al. 2005; Näsholm et al. 1998; Schimel and Chapin 1996; Schmidt and Stewart 1999). It seems that in most forest soils the organic N in solution can be a very essential nitrogen form for plant uptake, microbial transformation, and leaching. Unfortunately, this aspect receives relatively little attention compared with soil inorganic N.

Soil SON is generally defined as organic forms of N extracted by water or salt solutions ($CaCl_2$, KCl, K_2SO_4, etc.), while the DON is defined as organic N in soil solution and is usually measured by leaching method or suction cups (Qualls et al. 2000; Murphy et al. 2000). Although very few studies were related to the relationships between SON and DON, theoretically, it could be assumed that the SON may be the potential source for DON. Therefore, SON and DON should be closely correlated.

Soil SON can be derived from decomposition of soil organic matter, rhizo-deposition, desorption from soil colloids and microbial debris and atmospheric deposition (Marschner 1995; Pacheco et al. 2004; Qualls 2000). The amounts of SON in soils vary greatly with soil type, vegetation cover, management practice, environmental conditions (e.g. rainfall) and analytical methods used (Chapman et al. 2001; Hannam and Prescott 2003; Murphy et al. 2000; Qualls 2000; Zhu and Carreiro 2004; Chen et al. 2005). Although water extraction can cause the dispersion of clays and it can be difficult to obtain clean solution for analysis on the one hand, and salt extracts may disturb adsorption equilibrium on soil surface and release organic N which was not originally dissolved (Zsolnay and Goerlitz 1994; Murphy et al. 2000), some studies have been successfully carried out in relation to SON pools in forest soils. Most of those studies focused on the size of SON pools in forest soils in temperate ecosystems (Hannam and Prescott, 2003; Zhong and Makeschin, 2003; Zhu and Carreiro, 2004).

In this paper, the author used results from field and laboratory studies to approach the pool size and the relationships between SON and a series of other soil nitrogen indices, and to further identify the functions of SON pool in temperate forest soils in northeast China.

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Materials and methods

Site descriptions and soil sampling

Forty eight forest stands located at two sites in the temperate forests of northeast China were selected, encompassing the main forest and soil types of the region (Table 1). In each sampling stand, three cores (5 cm in diameter) of organic layer and mineral soil (0–3 and 3–15 cm, respectively, for free amino acid analysis in another paper; bulked as a composite sample for SON analysis in this paper) were randomly taken from an area of 20×20 m^2 in May 2006. This sampling time was just after the freeze-thaw season, and it is expected that at this time the soil SON pool in different stands would be relatively large and constant, so that the data could be precisely compared among forest types or between sites. Soil samples were packed in plastic bag and transported in a cooler with ice packs. Living vegetation debris were separated from samples by hand, organic layer samples were then snipped to less than 5 mm, and mineral soils freshly sieved through a 2 mm mesh. All samples stored at 4°C prior to analysis of extractable organic and inorganic N and microbial biomass N. A subsample of each soil was air-dried and stored at room temperature for analysis of soil basic chemical properties.

Table 1. Site characters as described with forest and soil types

Site location	Forest type	Soil classification by CST	Number of stands selected for sampling
Liangshui Nature Reserve, central Less Xingan Mountain (128°48′–128°55′ E, 47°07′–47°14′ N)	Virgin *Pinus koraiensis* forest	Bori-Udic Cambosols	6
	Virgin *Picea koraiensis* forest	Molli-Orthic Gleyosols	6
	Virgin *Abies nephrolepis* forest	Molli-Orthic Gleyosols	6
	Secondary broadleaved forest dominated by *Betula platyphylla*	Bori-Udic Cambosols	6
Maoershan Experimental Forest Farm (127°31′–127°34′ E, 45°21′–45°25′ N)	Secondary broadleaved forest dominated by *Betula platyphylla*	Bori-Udic Cambosols	6
	38-years-old *Larix gmelinii* plantation	Bori-Udic Cambosols	6
	16-years-old *Fraxinus mandshurica* plantation	Bori-Udic Cambosols	6
	49-years-old *Pinus koraiensis* plantation	Bori-Udic Cambosols	6

Analysis of soil SON and SIN

Water-extractable organic N is specially dealt with in the present study, since it might probably be the most exact indicator for SON. Water extracts were prepared by mixing 10 g (dry weight equivalent) of field moist soil samples with 100 ml of distilled water (soil: water ratio 1:10), shaking on ice for 1 h, and then gravity-filtering at 4°C through a quantitative analysis filter paper. All the procedures were controlled at low-temperature to minimize microbial activity and N mineralization during extraction and filtration. Extracts were stored at 4°C until all samples had been extracted and gravity-filtered (within 48 h). Each gravity filtrate was then vacuum-filtered through a 0.45 μm membrane filter and stored at –20°C prior to analysis.

Duplicate 5-ml aliquot were removed from the membrane filtrate and placed in acid-washed 50-ml glass vials for SON analysis; nitrate and ammonium concentrations in the remaining filtrate were determined using a Flow Injection Analyzer (Sweden5020). A modified persulphate solution was used to convert dissolved N in filtered soil extract to nitrate (Cabrera and Beare 1993). Vials were sealed with Teflon-lined caps, weighted, and autoclaved at 120°C for 45 min. After autoclaving, each vial was reweighted to determine evaporation loss and then diluted with 5 ml of distilled water. Nitrate concentrations in the persulphate digests were measured by UV Spectrophotometer (Yuzs et al., 1994), and SON was calculated by subtracting the quantity of SIN (nitrate plus ammonium) in the extract from the quantity of total soluble N (as nitrate).

Analysis of microbial biomass N

Soil microbial biomass N (MBN) was measured by the chloroform fumigation-extraction method (Vance et al., 1987): briefly, two 10 g (dry weight equivalent) fresh soil were weighed, one immediately extracted with 100 ml of 0.5 M K_2SO_4, another extracted after 24 h fumigation with ethanol-free chloroform in a desiccator. Total extracted N was determined as described above. Nitrogen released by chloroform fumigation (i.e. microbial N) was calculated by subtracting the total soluble N in unfumigated soil from the total soluble N in fumigated soil. A correction factor (K_{EN}) of 0.54 (Brookes et al. 1985; Joergensen and Mueller 1996) was used in the biomass N calculation.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2000 and SPSS version 13.0.

Results and discussion

Water extractable organic N

The water extractable SON ranged from 156.0 to 292.6 $mg \cdot kg^{-1}$ in organic layer and from 58.6 to 137.4 $mg \cdot kg^{-1}$ in 0–15cm mineral soil, with average concentrations of 200.4 $mg \cdot kg^{-1}$ in organic layer and 87.9 $mg \cdot kg^{-1}$ in 0–15cm mineral soil, respectively (Table 2). One-way analysis of variance (ANOVA) indicated that

the SON content in organic layer is significantly higher than that in 0–15cm mineral soil. Variable amounts of SON were present in soils of different forests, with significant differences among most of the forest types at the two sites (Table 2). Significant difference between the two secondary broadleaved forests located at the Liangshui Nature Reserve and the Maoershan Experimental Forest Farm were even found, implying that the influences of site condition and management level on soil SON should be quite considerable. The coefficient of variance (CV) among replicates within same study forest was in the range 15.5%–42.7%, which represents the influence of random factors on soil SON content.

The SON in water extracts comprised 73.5%–88.7% of total soluble N (TSN) and 0.66%–1.82% of total N (TN) in organic layer, and 82.4%–92.4% of TSN and 1.23%–2.62% of TN in 0–15cm mineral soil (Table 2). These calculations accounted for 68.8%–306.2% of microbial biomass N (Table 3), and 4.7–12.0 times higher than mineral N (data not shown).

Table 2. Soluble organic nitrogen (SON) extracted by water in temperate forest soils of northeast China (n =6)

Site	Forest	Soil SON					
		Organic layer			Mineral soil (0-15cm)		
		(mg· kg^{-1})	%(TSN)[a]	%(TN)[b]	(mg· kg^{-1})	%(TSN)[a]	%(TN)[b]
Liangshui Nature Reserve	Virgin *Pinus koraiensis*	196.0 c	86.6 a	1.13 b	87.1 c	84.8 b	1.89 bc
	Virgin *Picea koraiensis*	257.9 b	85.8 a	1.82 a	137.4 a	90.0 a	2.62 a
	Virgin *Abies nephrolepis*	189.4 c	75.1 b	1.54 b	99.5 bc	82.4 b	1.25 e
	Secondary broadleaved	292.6 a	88.7 a	1.41 b	106.5 b	85.4 b	2.12 b
Maoershan Experimental Forest Farm	Secondary broadleaved	160.1 d	88.4 a	0.75 c	72.4 de	91.2 a	1.67 c
	L. gmelinii plantation	162.4 d	82.8 a	1.19 b	58.6 f	90.3 a	1.23 e
	F. mandshurica plantation	156.0 d	73.5 b	0.66 c	75.8 d	92.4 a	1.43 d
	P. koraiensis plantation	188.8 c	84.5 a	1.38 b	65.5 ef	90.1 a	1.72 c
Mean		200.4	83.2	1.24	87.9	88.3	1.74

Values in the table are presented as mean of 6 replicates, data in the same column followed by different letters are significantly different (P<0.05). [a] Percentage of SON over total soluble nitrogen (TSN); [b] Percentage of SON over soil total nitrogen (TN).

The amount of soil SON has been investigated in some temperate forests and in certain subtropical areas. In temperate forest ecosystems, concentrations of SON extracted by various methods from organic layer generally ranged from 50 to 300 mg·kg^{-1} (Zhong and Makeschin, 2003; Hannam and Prescott 2003; Berthrong and Finzi 2006), and ranged from 6.5 to 23.6 mg·kg^{-1} in surface soils (0–15 cm or 0–10 cm) (Hannam and Prescott, 2003; Zhong and Makeschin 2003; Zhu and Carreiro 2004; Berthrong and Finzi 2006). A recent study on surface forest soils of subtropical Australia reported that the concentrations of SON extracted by 2 M KCl, 0.5 M K_2SO_4 and water was 5–45 mg·kg^{-1}, 2–42 mg·kg^{-1} and 1–24 mg·kg^{-1}, respectively (Chen et al. 2005). Water extracted SON from soils is less efficient than salt solutions due to the fact that salt solutions have greater potential for cation/anion exchange reactions leading to liberate physically adsorbed SON from clay minerals and/or soil organic matter, and the SON extracted by water was generally 20%–40% (about one-third on average) of SON extracted by salt solutions (Hannam and Prescott 2003; Willett et al. 2004; Chen et al. 2005). The water soluble SON pools in organic layers measured in this study were within the similar magnitude of salt solution extracted SON pools reported in the literature as above, while the water soluble SON in 0–15 cm mineral soils here were much higher (3–10 times) than any other reported soils, irrespective of forest and soil types and extraction methods (water or salt solutions), as discussed above. It is suggested that the potential sources of the SON in forest mineral soils included: 1) leaching of soluble organic matter and microbial biomass (e.g. shelf fungi) from forest floors (leaf litter, woody debris, etc.) and from tree canopy; 2) microbial dissolution of soil organic matter (e.g. cellulose, lignin); 3) microbial debris and metabolites; 4) root exudation and turnover; and 5) atmospheric organic N deposition (Kalbitz et al. 2000; Qualls et al. 2000, 2002; Neff et al. 2003; Cornell et al. 2003; Pacheco et al. 2004; Chen et al. 2005). On a comprehensive consideration of the most probable factors, the abnormal abundance of soil SON in the present study might have resulted from: i) the 0–15cm mineral soils in this study were typically characterized by high organic matter and high organic nitrogen content (3.81–7.95g·kg^{-1}), which might have brought high SON consequently; ii) the sampling date was just after the freeze-thaw season and the temperature of mineral soils was still too low to generate a soil microbial flush or enable any significant activity of soil enzymes responsible for SON transformation, thus, some organic nitrogen leached from organic layer, or released by freeze-thaw events (including low molecular humic nitrogen and bio-nitrogen) might temporarily accumulate as "soluble" in surface mineral soils.

The spacial variation in SON pools observed in the present study was analogical to many other reports (Hannam and Prescott 2003; Zhong and Makeschin 2003; Willett et al. 2004; Chen et al. 2005; Berthrong and Finzi 2006), and it could be attributed to a combination of factors including soil types (N quantity and quality), tree species (leaf and root litters), management levels and environmental conditions (temperature and moisture).

Microbial biomass N

Microbial biomass N (MBN) in the 0–15cm mineral soil varied markedly among different forests, ranging from 32.5 to 105.2 mg·kg^{-1} (Table 3). Differences among many of the forest types at

the two sites were statistically significant (Table 3). The coefficient of variance (CV) across replicates within same study forest was in the range 17.5%–37.8% (date not shown), suggesting a moderate influence of random factors on soil MBN pool size.

The MBN pools in surface mineral soils measured in this study were comparable to or moderately larger than that reported in literatures (Zhong and Makeschin, 2003; Chen et al. 2005; Berthrong and Finzi, 2006; Christoua et al. 2006; Yang et al. 2007). On the other hand however, these values only accounted for 1.44% of soil total N on average (Table 3), a quite low percentage compared with some other studies on temperate or subtropical forest soils which reported MBN comprised 2.8%–5.2% of total N (Zhong and Makeschin, 2003; Chen et al. 2005). Considering the sampling date was just after the freeze-thaw season and the temperature conditions of mineral soils were still insufficient for soil microbes to flourish, the MBN reported here might be conservative estimates of the "actual" MBN pools that should present in growing seasons.

Table 3. Microbial biomass N (MBN) in temperate forest soils of northeast China (n =6)

Site	Forest	MBN in 0-15cm mineral soil	
		($mg \cdot kg^{-1}$)	%(TN)[a]
Liangshui	Virgin *Pinus koraiensis*	72.7 b	1.58 bc
Nature Re-	Virgin *Picea koraiensis*	47.3 c	0.90 d
serve	Virgin *Abies nephrolepis*	32.5 d	0.41 e
	Secondary broadleaved	93.2 a	1.85 b
Maoershan	Secondary broadleaved	105.2 a	2.43 a
Experimental	*L. gmelinii* plantation	61.6 b	1.29 c
Forest	*F. mandshurica* plantation	98.5 a	1.87 b
Farm	*P. koraiensis* plantation	45.8 c	1.20 c
Mean	-	69.6	1.44

Values in the table are presented as mean of 6 replicates, data in the same column followed by different letters are significantly different ($P < 0.05$).

[a] Percentage of MBN over soil total nitrogen (TN).

Relationships between soluble organic N, microbial N and other soil N indices

Across all the replicates of all studied forests at the two sites of Liangshui Nature and Maoershan Experimental Forest Farm, water extractable SON in 0–15cm mineral soil was positively and significantly correlated with total soluble N (TSN), soil total N (TN) and organic matter content (OM), while significant positive correlations among many other soil N indices were also observed (Table 4). Little correlation was found between SON and soluble mineral N (SIN) (r = 0.071). Unexpectedly, the correlations between microbial biomass N (MBN) and SON, TSN, or OM were all negative, though having not reached a statistically significant level except that between MBN and OM. (Table 4).

It has been found that SON is significantly correlated with soil total N in temperate and subtropical forest soils (Zhong and Makeschin 2003; Zhu and Carreiro 2004; Chen et al. 2005), supporting that soil native organic N is one of the main source of SON. Microbial decomposition of soil organic matter (including SOC, SON) is considered to be another major factor controlling the amount of soluble organic matter retained in soil (Kalbitz et al., 2000; Qualls et al. 2002). In support of that MBN may act as an intermediate sink and source for SON (Kalbitz et al. 2000; Qualls et al. 2002; Qualls and Richardson, 2003), it has also been reported that the SON in salt solution or water extracts was significantly correlated with MBN (Zhong and Makeschin 2003; Chen et al. 2005). In our study, the high SON in mineral soils in early spring might include the contribution of MBN that released from "the mass dying of microbes from winter freeze", and this was supported by corresponding high concentrations of free amino acids (to be reported in another paper); but the negative SON-MBN correlation might merely caused by the relatively lower soil microbial biomass in *Abies* and *Picea* forests where the soil temperature were particularly low because of shading and low-lying, whether it essentially means a net sink at the very time is still in the open. In fact, it is still very obscure whether MBN is a source or a sink for SON in forest ecosystems, and direct evidence supporting this is still lacking. Perakis and Hedin (2001), in applying ^{15}N pool dilution and pulse-chase tracer techniques to investigate the fate of N in unpolluted old-growth temperate forest soil, suggested that ^{15}N loss from microbial biomass did not enter the extractable pool of dissolved organic N (DON) and that DON losses did not originate directly from "active" microbial turnover. Therefore, the "source-sink relations" between MBN and SON in forest ecosystems and its quantification exactly need further investigation (Chen et al., 2005). In addition，just as has been recognized that the SON can be directly produced by microbial turnover (e.g. free amino acid release) and indirectly through microbial generation of extracellular enzymes (e.g. proteolysis) (Neff et al., 2003), it can also be directly consumed by active microbes (e.g. uptake of free amino acids and amino sugars) and indirectly through extracellular enzymes (e.g. humification), and therefore, the activity of extracellular enzymes and the environmental factors (especially severe conditions such as freeze-thaw) must be take into account when the interrelations between SON and MBN are to be investigated.

Table 4. Results of correlation analysis between SON and other nitrogen forms in the 0-15cm mineral soil, across all the replicates of all studied forests at the two sites of Liangshui and Maoershan (n =48)

Pearson r	SON	SIN	TSN	MBN	AHN[a]	TN	OM[b]
SON	1						
SIN	0.071	1					
TSN	0.723**	0.397*	1				
MBN	−0.187	−0.175	−0.222	1			
AHN[a]	0.223	0.185	0.287*	0.087	1		
TN	0.327*	0.316*	0.420**	−0.189	0.367*	1	
OM[b]	0.356*	0.103	0.511**	−0.286*	0.309*	0.526**	1

* Significant at the 0.05 level; ** Significant at the 0.01 level; [a] Alkali hydrolysable N determined with 1.8 $mol \cdot L^{-1}$ NaOH; [b] Organic matter content.

Conclusion

The water extracted SON pools in the organic layer of temperate forest soils in northeast China were measured within the similar magnitude of salt solution extracted SON pools reported in literatures, while the water soluble SON in surface mineral soils here were much higher (3–10 times) than any other reports. Water soluble SON varied markedly among the soils under different forests and at different sites. The SON in water extracts were positively and significantly correlated to soil organic matter and total N contents, but negatively correlated to microbial biomass N. There are still many gaps in our understanding of how SON is produced, retained, and transformed in forest soils, and the chemical and biological nature and dynamics of these SON pools remain poorly understood too. All these aspects need to be further studied.

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